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DROUGHT STRESS

Determination of Moisture Deficit and Heat Stress Tolerance in Corn Using Physiological Measurements and a Low-Cost Microcontroller-Based Monitoring System

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Abstract

In the southern United States, corn production encounters moisture deficit coupled with high-temperature stress, particularly during the reproductive stage of the plant. In evaluating plants for environmental stress tolerance, it is important to monitor changes in their physical environment under natural conditions, especially when there are multiple stress factors, and integrate this information with their physiological responses. A low-cost microcontroller-based monitoring system was developed to automate measurement of canopy, soil and air temperatures, and soil moisture status in field plots. The purpose of this study was to examine how this system, in combination with physiological measurements, could assist in detecting differences among corn genotypes in response to moisture deficit and heat stress. Three commercial hybrids and two inbred germplasm lines were grown in the field under irrigated and non-irrigated conditions. Leaf water potential, photosynthetic pigments, cell membrane thermostability (CMT) and maximum quantum efficiency of photosystem II (Fv/Fm) were determined on these genotypes under field and greenhouse conditions. Variations observed in air and soil temperatures, and soil moisture in plots of the individual corn genotypes helped explain their differences in canopy temperature (CT), and these variations were reflected in the physiological responses. One of the commercial hybrids, having the lowest CT and the highest CMT, was the most tolerant among the genotypes under moisture deficit and heat stress conditions. These results demonstrated that the low-cost microcontroller-based monitoring system, in combination with physiological measurements, was effective in evaluating corn genotypes for drought and heat stress tolerance.

Introduction

Moisture deficit accompanied by high-temperature stress is a major abiotic stress factor that affects corn production in the southern United States. In the Mid-South, corn plants frequently encounter a period of drought and heat stress during flowering and kernel development (Bruns 2005), causing damage to the crop.

Various physiological traits related to drought and heat stress have been used to monitor responses of plants to these environmental conditions. At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and associated pigments, chlorophyll and carotenoids (Iturbe-Ormaetxe et al. 1998), which are mainly involved in harvesting light and generating reducing powers in the form of NADPH (Garrett and Grisham 2005). In addition to their well-established function as collectors of light energy for photosynthesis, carotenoids are responsible for scavenging triplet chlorophyll and singlet oxygen and protecting the photosynthetic apparatus from oxidative stress (Havaux 1998, Camejo et al. 2005, Balouchi 2010). Loss of these pigments during

environmental stress is a good indicator of the response of plants to the particular stress, and change in chlorophyll/carotenoid ratio is considered to be a sensitive indicator of oxidative damage (Hendry and Price 1993). The maximum photosystem II (PSII) efficiency (Fv/Fm) can also be used as an indicator of changes in the photosynthetic apparatus as the plants' environment changes. Chlorophyll fluorescence is a quick and non-destructive technique widely used to investigate damage to PSII and thylakoid membrane by various types of stresses including heat and drought (Krause and Weiss 1984, Camejo et al. 2005, Ristic et al. 2007, Tang et al. 2007, Wahid et al. 2007).

Cellular membranes are among the first targets of many plant stresses, and it is generally accepted that the maintenance of their integrity and stability under stress conditions is a major component of tolerance (Levitt 1980, Bajji et al. 2001). Under stress conditions, membrane fluidity changes, lipid peroxidation increases and membrane selectivity is often impaired (Levitt 1980, Su et al. 2007, Dias et al. 2010). Measurement of cell membrane thermostability (CMT) as the conductivity of electrolytes leaking from tissues is one technique that has been used as an indirect method of screening for heat stress tolerance in various crop species including corn (Ismail and Hall 1999, Srinivasan et al. 1996, Tang et al. 2007, Dias et al. 2010).

The amount of water used by a crop plant at any time depends, among other things, on moisture availability in the soil, air temperature and soil temperature. Determination of soil moisture status is of major consideration regarding plant water relations. Soil temperature is also important as extreme soil temperatures can limit the availability of water to the roots and cause water deficit stress (Gavito et al. 2001, Lambers et al. 2008). Canopy temperature (CT) measurement relative to ambient air temperature is often used to assess plant stress arising from moisture deficit or high temperature (Gonzalez-Dugo et al. 2006, Reynolds et al. 2007) and has been used in identifying genotypes that maintain lower CT as compared to other genotypes under the same moisture deficit or heat stress conditions (Balota et al. 2008, Kashiwagi et al. 2008, Rashid et al.1999). A relatively lower canopy temperature in drought stressed plants indicates a better capacity for taking up soil moisture and maintaining a relatively better plant water status (Cure et al. 1989).

In the field, crops are routinely subjected to a combination of different abiotic stresses such as limited water availability, which is frequently associated with high temperature (Jiang and Huang 2001, Rizhsky et al. 2002, Mittler 2006). A combination of drought and heat stress has a greater detrimental effect on the growth and productivity of plants compared with each of the stresses applied individually. Recent studies have revealed that the response of plants to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response of plants to each of the different stresses applied individually (Mittler 2006, Rizhsky et al. 2002). To have a better understanding of how plants respond to environmental stress, we need to monitor and record changes in their physical environment under natural conditions, especially when there are multiple stressors, and integrate this information with their physiological responses. A low-cost microcontroller-based monitoring system was developed to automate the measurement and recording of canopy, soil and air temperatures, and soil moisture status in cropped fields, which among other factors, affect plant and soil water relations (Fisher and Kebede 2010). This automated system records data continuously throughout the cropping season and during inclement weather when manual measurements would likely not be collected. Sensors installed in a fixed location ensure consistency in measurements (same location in the field, same part of the plant and same physical orientation of the sensors). The purpose of this study was to examine how our automated system, in combination with physiological measurements, can help monitor moisture deficit and heat stress and detect differences among corn genotypes in response to these stresses.

Materials and Methods

Plant material and experimental design

This study was conducted at the Jamie Whitten Delta States Research Center, Stoneville, MS, UDSA-ARS, in 2009 and 2010. The soil at the experimental site was a Beulah fine sandy loam (coarse-loamy, mixed thermic Typic Dystrochrepts). Three corn commercial hybrids and two germplasm lines were planted on 7 April 2009 and 6 April 2010. The hybrids consisted of Pioneer 31G70, Pioneer 32B34 and DeKalb 63-42. The germplasm lines were developed by the Germplasm Enhancement of Maize (GEM) Programme in 2003 and 2004 (USDA-ARS, Ames, IA, USA) with tropical background: PI 489361 (GEMS-0092, a drought stress-tolerant inbred germplasm with 25 % tropical background from Cuba) and PI 639055 (GEMS-0030, aflatoxin resistant inbred germplasm with 50 % tropical background from Brazil). For the convenience of illustration, Pioneer 31G70, Pioneer 32B34, DeKalb 63-42, PI 639055 and PI 489361 will be designated as Hybrid 1, Hybrid 2, Hybrid 3, Germ 1 and Germ 2, respectively (Table 1).

The experiment was conducted under two soil moisture treatments. A split-plot experimental design was used with main unit treatments consisting of two soil moisture treatments, irrigated and non-irrigated, and five corn

Table 1 Corn genotypes used in the study and their designation

Genotype	Designation
Pioneer 31G70 – Commercial hybrid	Hybrid 1
Pioneer 32B34 – Commercial hybrid	Hybrid 2
DeKalb 63-42 – Commercial hybrid	Hybrid 3
PI 639055 (GEMS0030) – Inbred germplasm	Germ 1
PI 489361 (GEMS-0092) – Inbred germplasm	Germ 1

genotypes as subplot treatment. Main unit experimental design was a randomized complete block with four blocks, and each main treatment was randomly assigned within each block. Subunit treatments were randomly assigned within each main unit treatment and arranged in plots each consisting of four rows of 9.1 m long, with 1 m row spacing, and planted at a seeding rate of about 70 000 seeds ha^{-1}. Non-irrigated buffer strips consisting of four rows were planted between and parallel to all irrigated and non-irrigated main unit treatments. Prior to planting, K as muriate of potash at 67 kg ha^{-1} and N as NH_4NO_3 at 112 kg ha^{-1} were applied and incorporated into the soil. Additional N, in a liquid fertilizer form at 100 kg ha^{-1}, was applied at growth stage V6. Irrigation treatments were applied beginning at anthesis (early June) using furrow irrigation with a 10-day rotation between irrigations or after a rain event of 25 mm or more, which is a schedule commonly used for corn production in the region after silking.

Moisture and temperature data collection using an automated microcontroller system

Measurements of leaf, air, and soil temperatures and soil moisture status were made using an automated microcontroller-based monitoring system. The monitoring system, described in detail by Fisher and Kebede (2010), consisted of electronic sensors and a microcontroller-based circuit. Thirty of the data-collection systems were installed in plots consisting of five genotypes and two soil moisture treatments with three replications of each combination. The temperature sensors consisted of an infrared thermometer sensor for measuring plant canopy temperature, a digital sensor for measuring soil temperature and an analog sensor for measuring air temperature. Soil moisture status was determined using a Watermark 200-SS (Irrometer, Riverside, CA, USA) water-potential sensor.

In each plot, the monitoring systems were installed in one of the two middle rows of a four-row plot, halfway down the length of the plot. Soil-moisture and soil-temperature sensors were installed at a depth of 30 cm below the soil surface. The infrared leaf-temperature sensor was installed inside a thick-walled PVC plastic enclosure and attached with a clamp to a tall fibreglass pole so that the height of the sensor could be adjusted periodically as the plants grew. The sensor was oriented facing north, aimed at the south-facing leaves of the crop. The air-temperature sensor was mounted in the canopy approximately 30 cm above the soil surface and shaded to avoid direct exposure to sunlight.

Throughout the growing season, data were downloaded during periodic visits to each location to a handheld computer. The data, in standard ASCII text format, were later transferred to a desktop computer for examination, analysis and storage.

Physiological measurements

Chlorophyll and carotenoids

Chlorophyll and carotenoids were determined on leaves in about 10-day intervals starting at anthesis until late dent stage (from the beginning of June to the end of July). Leaf samples were cut from the middle part of the blade, placed in plastic bags and kept on ice in a cooler for transport to the laboratory. Two 10-mm diameter leaf discs were taken from each leaf sample and placed in a vial containing 2 ml of absolute ethanol and incubated for 24 h at room temperature (25 °C) in the dark. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids were determined by measuring absorbance at 480, 645 and 663 nm wavelengths on a spectrophotometer (Beckman Coulter DU 800 Spectrophotometer, Brea, CA, USA) and computed following the method of Hendry and Price (1993). Then, total chlorophyll (Chl), chlorophyll *a*/chlorophyll *b* ratio (Chl *a*/*b*) and chlorophyll/carotenoid ratio (Chl/Carot) were calculated. Fifteen 16-mm diameter leaf discs were punched from the same leaf samples to determine specific leaf weight. The leaf discs were dried at 70 °C for 72 h, and specific leaf weight was calculated as dry weight per unit leaf area.

Leaf water potential

Leaf water potential (Ψ_w) was determined on the same leaves used for chlorophyll determination using leaf cutter thermocouple psychrometers (J.R.D. Merrill Specialty Equipment, Logan, UT, USA) at midday (1200–1300 h). A 5-mm diameter leaf disc was taken from the midpoint along the length of the leaf blade and placed in a leaf cutter thermocouple psychrometer. Samples were taken from four individual plants for each genotype per water treatment in four replications. The leaf cutter thermocouple psychrometers were placed in a water bath at 25 °C for 4 h. Outputs from the psychrometers were recorded by a PSYPRO data logger (WESCOR Inc., Logan, UT, USA). Three Ψ_w readings were taken from each sample, and the average of the three readings was calculated for each of the four samples per plot.

Cell membrane thermostability and chlorophyll fluorescence

To complement the field experiment, plants from the same corn genotypes were grown in a greenhouse in 19-l pots containing Metro-Mix 200 (Sun Gro, Bellevue, WA, USA) in six replications under a 28/23 °C day/night temperature regime and 16-h photoperiod (with supplemental lighting). The corn seedlings were watered every day with a half-strength Hoagland's nutrient solution. Pots were rotated periodically to minimize position-induced plant-to-plant variation. When the plants were about 5 weeks old, temperature in the greenhouse was raised to 38/33 °C day/night for 7 days to impose heat stress on the plants. Before the heat stress treatment was imposed, initial (no heat stress) chlorophyll fluorescence (CF) measurements were made on the youngest and fully expanded leaves. The leaves were dark adapted using dark-adaptation clips for 1 h, and CF (Fv/Fm- variable fluorescence/maximal fluorescence) was measured using an OS1-FL modulated chlorophyll fluorometer (Opti-Sciences, Hudson, NH, USA). CF measurements were made on the heat-stressed plants on the third, fifth and seventh day after the start of the heat stress treatment. Immediately after each CF measurement, leaf discs were taken in the same leaf blade area that was used for CF to determine chlorophyll content and CMT. For CMT determination, a cork borer with 10-mm inner diameter was used to collect 10 leaf discs from each leaf used for CF. The leaf discs were immediately placed in glass vials containing 2 ml deionized water and quickly brought to the laboratory. Leaf discs were thoroughly rinsed three times in deionized water to wash out any adherent electrolytes from both leaf surfaces and damaged cells because of cutting. After final washing, 10 ml of water was added to each tube, and samples were incubated at room temperature (25 °C) for 24 h. To determine electrolyte leakage on the samples, electrical conductivity (EC) was measured after 24 h (T1) using a Solution Analyzer 4603 (Amber Science Inc., Eugene, OR, USA). The samples were then autoclaved for 10 min at 0.10 MPa pressure to completely kill the cells and release all the electrolytes. Vials were brought to 25 °C and final EC was measured (T2). Percentage relative cell injury (RCI %), an indicator of CMT, was calculated with the following formula:

$$\mathrm{RCI}\,(\%) = 1 - \frac{1 - \left(\frac{T1}{T2}\right)}{1 - \left(\frac{C1}{C2}\right)} \times 100$$

where T and C refer to EC values of heat-treated and control leaf discs, and 1 and 2 denote initial and final EC readings, respectively (Sullivan 1972).

Additional CMT determination was made on leaf samples collected from the field plants. Heat stress was induced by incubating leaf discs at 40 °C for 6 h. Measurements on EC were made at 1.5, 3.0, 4.5 and 6.0 h during the incubation period. After 6 h, the samples were autoclaved to measure final EC.

Statistical analysis

All field data collected on the corn genotypes under irrigated and non-irrigated conditions were analysed using a two-way PROC ANOVA procedure in SAS ver. 9.2 (SAS Institute, Cary, NC, USA) to test differences among the genotypes under the two soil moisture treatments. Cell membrane thermostability and chlorophyll fluorescence data were analysed using PROC MIXED procedure in SAS to detect differences in measurements over time at $\alpha = 0.05$ significance level. Pearson's correlation test was done on the data from the microcontroller-based monitoring systems and the physiological measurements. The data used from the microcontroller systems (soil water potential, and soil, air and canopy temperatures) were the maximum values of the hourly measurements on the seven sampling dates used for the physiological measurements.

Results

Weather conditions

The corn-growing season for 2010 was hotter and drier than that of the 2009 season at Stoneville, MS. The monthly average maximum temperatures for the months of April, May, June, July and part of August were 24.1, 26.9, 33.4, 32.2 and 33.3 °C (Aug 1–19) for 2009, and 26.8, 30.1, 34.4, 34.0 and 38.1 °C for 2010, respectively (from Mississippi State University weather network) (Fig. 1). Total precipitation for the 2009 growing period was 666 mm whereas it was only 266 mm for 2010. Monthly distribution was 58.2, 343.2, 6.9, 222 and 36.1 mm for 2009, and 46.2, 134.6, 31.5, 48 and 61 mm for 2010 for the months of April, May, June, July and August, respectively (Fig. 1). The monthly rainfall distribution was much lower in 2010 except for the month of June, which was the driest month in both seasons. July was hotter and drier in 2010 than in 2009, which brought most of the difference in physiological measurements between the 2 years.

Data from the microcontroller-based monitoring systems

Data on leaf, soil and air temperatures and soil water potential were collected hourly in the corn plots starting at seedling (five leaf stage) through harvest using the thirty microcontroller systems under irrigated and non-irrigated conditions. An example of hourly measurements from irrigated and non-irrigated plots over a 4-day period in July 2010 is shown in Figure 2a–c. Soil moisture measurements from mid-May until harvest are shown in Figure 2d. There

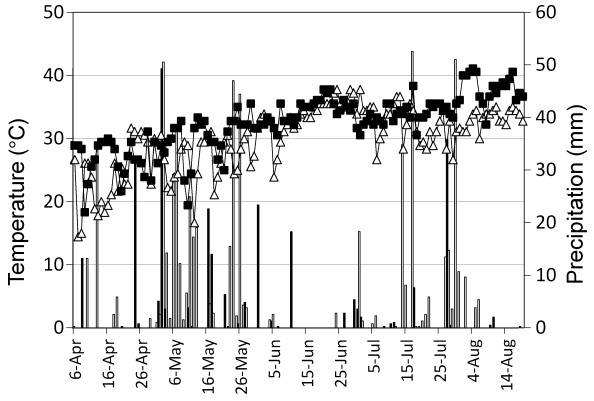

Fig. 1 Maximum daily temperature in °C (open triangles and solid squares for 2009 and 2010, respectively) and precipitation in millimetre (open bars and solid bars for 2009 and 2010, respectively) in Stoneville, MS, during the growing period of the corn genotypes in this study (Source: Mississippi State University, Delta Weather Station, Stoneville, MS).

were no differences in these four parameters between the irrigated and non-irrigated treatments until irrigation started in early June. Maximum CT in the non-irrigated plots was 2–5 °C higher than in the irrigated plots, with highest temperatures ranging from 35 to 39 °C and 32 to 35 °C for the non-irrigated and irrigated plots, respectively. Air temperature in the canopy microclimate was also higher in the non-irrigated plots (Fig. 2b). Similarly, soil temperature at 30 cm depth was 3–5 °C higher in the non-irrigated plots (Fig. 2c). During this period, soil water potential ranged from 0 (right after irrigation) to −55 kPa (right before irrigation) in the irrigated plots and dropped as low as −230 kPa in the non-irrigated plots (Fig. 2d).

Differences were observed in CT among the corn genotypes in both irrigated and non-irrigated plots. Among the hybrids, Hybrid 3 followed by Hybrid 2 had higher CT under both soil moisture treatments (Fig. 2a). The two germplasm lines, Germ 1 and Germ 2, had much higher CT accompanied by higher soil and air temperatures compared to the commercial hybrids (Fig. 2b,c). Hybrid 1 had the lowest CT among all the genotypes.

Pearson's correlation test was performed on the data from the microcontroller-based monitoring systems and the physiological measurements to show the association between the two data sets (Table 2). As there was year × soil moisture treatment interaction, only the data from the non-irrigated treatment in 2010 were used to do the correlation test. This test may not be close to the actual correlation because the data used from the monitoring systems were maximum values of the hourly measurements on the seven sampling dates used for the physiological measurements. However, the correlation test

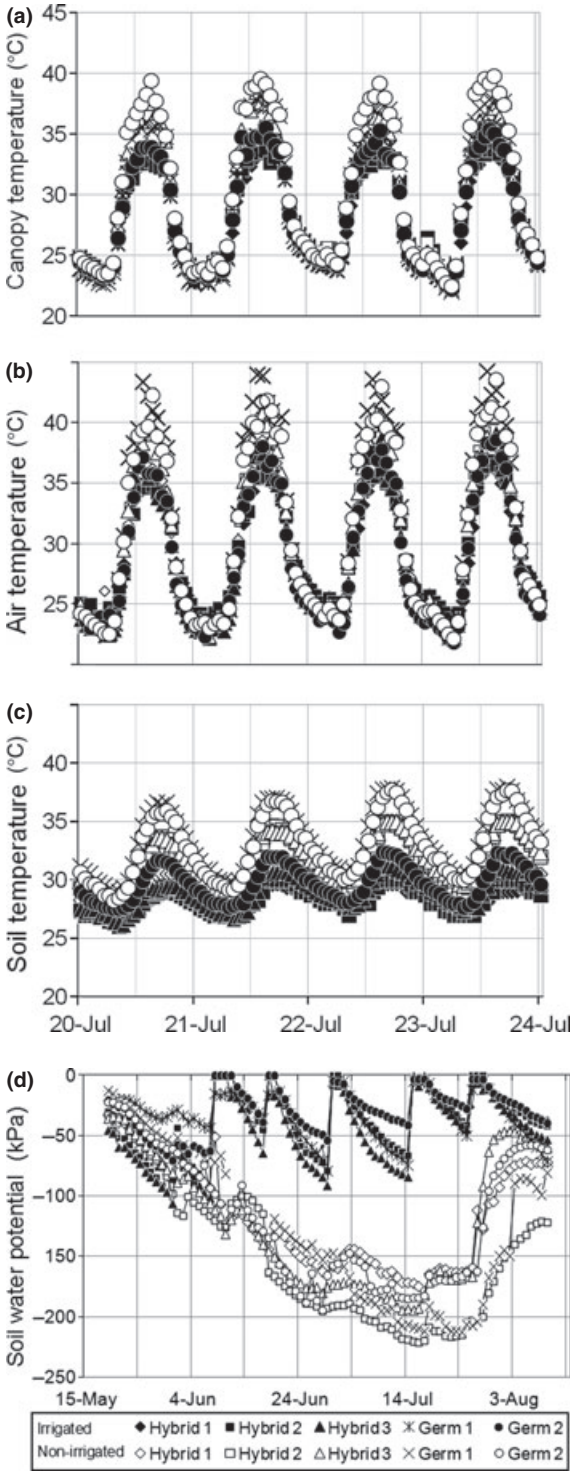

Fig. 2 A sample of hourly measurements of (a) canopy temperature, (b) air temperature and (c) soil temperature over a 4-day period in July 2010, and (d) soil water potential for the 2010 growing season in plots of five corn genotypes from irrigated and non-irrigated plots. Values for each genotype are means of three replicates.

Table 2 Correlation matrix of data from microcontroller-based monitoring system (soil water potential (SWP), soil temperature, air temperature and canopy temperature), and physiological measurements (leaf water potential (Ψ_w)), chlorophyll (Chl) and carotenoids (Carot)) under non-irrigated conditions in 2010; main entries are r-values, with P-values in parentheses

	SWP	Soil temperature	Air temperature	CT	Ψ_w	Chl	Carot
SWP	0.0000 (1.0000)						
Soil temperature	−0.4972 (0.0154)	0.0000 (1.0000)					
Air temperature	−0.2790 (0.3169)	0.8173 (0.0024)	0.0000 (1.0000)				
CT	−0.5014 (0.0113)	0.3940 (0.1718)	0.5805 (0.0218)	0.0000 (1.0000)			
Ψ_w	0.6550 (0.0080)	−0.3101 (0.1355)	−0.5687 (0.0145)	−0.4907 (0.0516)	0.0000 (1.0000)		
Chl	0.4337 (0.1394)	−0.1482 (0.3806)	−0.3940 (0.2601)	−0.3042 (0.1835)	0.8532 (0.0012)	0.0000 (1.0000)	
Carot	0.3755 (0.1756)	0.0765 (0.8704)	0.0434 (0.9255)	−0.4878 (0.1103)	0.8821 (0.0007)	0.9168 (0.0001)	0.0000 (1.0000)

Table 3 Mean values of chlorophyll *a* (Chl *a*), chlorophyll *b*, (Chl *b*), total chlorophyll (Chl), chlorophyll *a*/chlorophyll *b* ratio (Chl *a*/*b*), carotenoids (Carot), and chlorophyll/carotenoids ratio (Chl/Carot), plant height and specific leaf weight (SLW) from five field-grown corn genotypes under no moisture deficit or heat stress conditions (n = 12). Values are means of data from the 2009 and 2010 seasons

Genotype	Chl *a* (μmol cm^{-2})	Chl *b* (μmol cm^{-2})	Chl (μmol cm^{-2})	Chl *a*/*b*	Carot (μmol cm^{-2})	Chl/Carot	Plant height (cm)	SLW (mg cm^{-2})
Hybrid 1	21.9^{b}	7.0^{b}	28.9^{b}	3.13^{a}	8.8^{c}	3.28^{b}	278^{b}	1.28^{b}
Hybrid 2	22.3^{b}	7.0^{b}	29.3^{b}	3.19^{a}	8.9^{bc}	3.29^{b}	288^{a}	1.33^{b}
Hybrid 3	21.6^{b}	6.6^{b}	28.2^{b}	3.27^{a}	8.3^{c}	3.39^{a}	253^{c}	1.19^{c}
Germ 1	22.3^{b}	7.4^{b}	29.6^{b}	3.01^{a}	9.5^{b}	3.12^{c}	201^{d}	1.28^{b}
Germ 2	23.5^{a}	9.9^{a}	33.4^{a}	2.37^{b}	10.5^{a}	3.18^{c}	247^{c}	1.75^{a}
$LSD_{(0.05)}$	0.90	0.84	1.62	0.22	0.61	0.07	9.27	0.089
	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0003	P < 0.0001	P < 0.0001

Means with different letters in columns are significantly different.

still shows the trends in the relationships between the data from the monitoring system and the physiological measurements. Furthermore, significant correlations have been observed between the two data sets (Table 2).

Soil-plant water relations

Figure 3a shows soil water potential (SWP) measurements for irrigated and non-irrigated plots during the 2009 and 2010 growing seasons. Soil moisture was lower in 2010 than in 2009 as a result of less precipitation and higher temperature. Initial Ψ_w measured in early June, before irrigation treatments started, was lower in 2010 measuring −1.3 MPa compared to −1.0 MPa in 2009 (Fig. 3b), resulting from lower soil water potential (Fig. 3a). Leaf water potential decreased significantly under both soil moisture treatments in June with more negative values in the non-irrigated treatments. In July 2009, Ψ_w reached similar values in the two soil moisture treatments, whereas in 2010, Ψ_w continued decreasing in both soil moisture treatments until the last sampling date at the end of July with values as low as −2.28 MPa in the non-irrigated treatments. Correlation test showed that Ψ_w was significantly correlated with SWP (r = 0.6550, P < 0.0380) (Table 2). Leaf water potential also showed significant correlation with SWP and soil, air and canopy temperatures (Table 2). Differences in Ψ_w were not detected among the genotypes (P < 0.1260) because of large variability in values within each genotype.

Photosynthetic pigments

Chlorophyll and carotenoid contents determined on the same leaves used for Ψ_w analysis followed a similar pattern to the change in Ψ_w (Fig. 3c). As shown in Figure 3b,c, the changes in Ψ_w were associated with changes in Chl over the seven sampling dates in the two growing seasons. Highly significant positive correlation was observed between Ψ_w and the pigments (Chl (r = 0.8532, P < 0.0012; Carot (r = 0.8821, P < 0.0007)) (Table 2). Even though they were not significant, Chl showed positive correlation with SWP and negative correlation with air temperature and canopy temperature (Table 2). Similarly, carotenoids had positive correlation with SWP and negative correlation with canopy temperature. After irrigation started in early June, plants in the non-irrigated plots had lower Chl and Chl/Carot ratio, but had higher Chl *a*/*b* ratio compared to those in the irrigated plots in both years (Fig. 3c–e). There was a reduction in Chl and carotenoids in the samples analysed for the month of

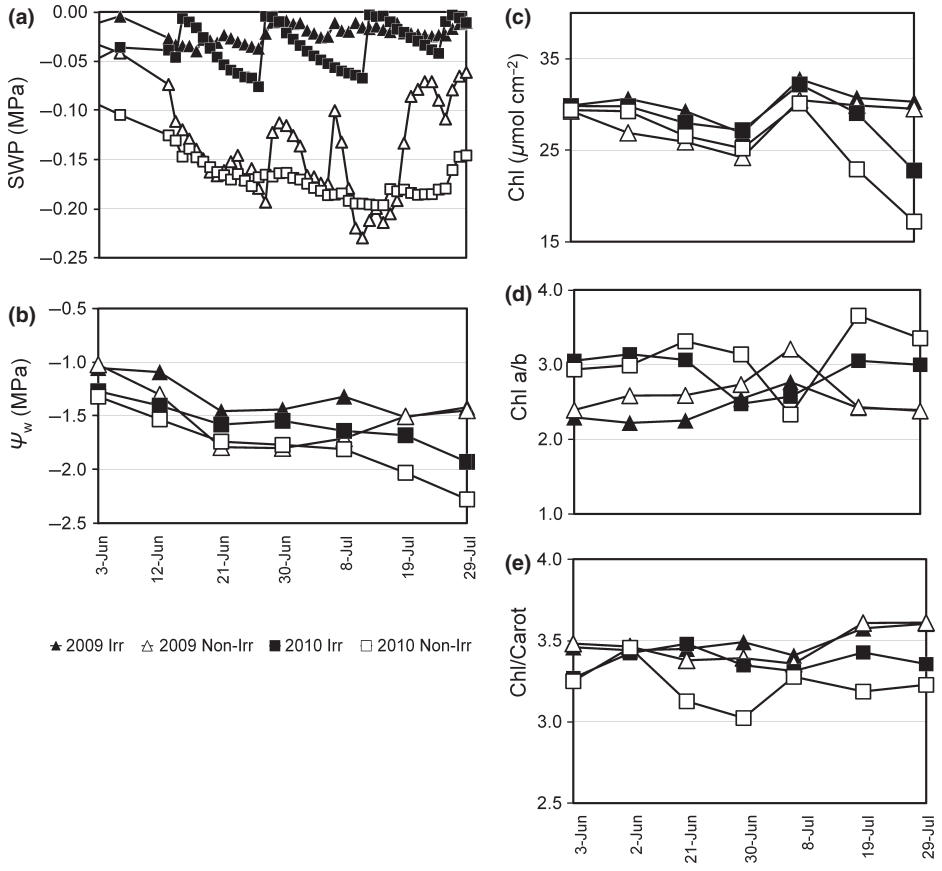

Fig. 3 Changes in soil water potential and its association with changes in leaf water potential and photosynthetic pigments: (a) soil water potential (SWP), (b) leaf water potential (Ψ_w), (c) total chlorophyll content (Chl), (d) chlorophyll *a*/chlorophyll *b* ratio (Chl *a*/*b*), and (e) chlorophyll/carotenoid ratio (Chl/Carot), in 2009 and 2010 on irrigated and non-irrigated corn plots. Data were collected on seven sampling dates from anthesis to late dent stage. Each data point is a mean value of five corn genotypes.

June for both years. As shown in Figure 1, during this period of time, there was little precipitation and air temperature was high. A slight increase in these photosynthetic pigments was observed in the samples taken in the early part of July, which could be due to the age of the leaves. During this period, the leaves being sampled were physiologically more mature than those sampled at the start and were gradually accumulating more Chl. In addition, there were several rain showers at the end of June and beginning of July in both years with slightly cooler temperatures. However, in the latter part of July, the pigments were much lower in 2010 than in 2009 (Fig. 3c). Statistical analysis on pigment content showed year × treatment interaction ($P < 0.0365$). Significant differences were also observed among the corn genotypes grown under the two soil moisture treatments in both years ($P < 0.0079$ for 2009 and $P < 0.0001$ for 2010). However, there was no genotype by soil moisture treatment interaction in all measurements in both years. Among the hybrids, Hybrid 3 had lower Chl *a*, Chl *b* and carotenoids in the majority of the sampling dates under both irrigated and non-irrigated conditions, but it had slightly higher Chl *a*/*b* ratio under non-irrigated treatments (Fig. 4). The two inbred germplasm lines had values at the highest and the lowest ends. Germ 2 had much higher Chl *a*, Chl *b* and carotenoids among all genotypes under both soil moisture treatment (in most cases at $P < 0.0001$), which could be attributed to its significantly higher specific leaf weight (Table 3), but had the lowest Chl *a*/*b* ratio. The reverse was true for Germ 1. It had the lowest Chl and carotenoid contents, but had a higher Chl *a*/*b* ratio among all genotypes under stress conditions.

Cell membrane thermostability and chlorophyll fluorescence

There was a significant reduction in CMT, Chl, and in dark-adapted chlorophyll fluorescence (Fv/Fm) when heat stress was imposed on the corn plants (38/33 °C day/night temperature) in the greenhouse for 7 days

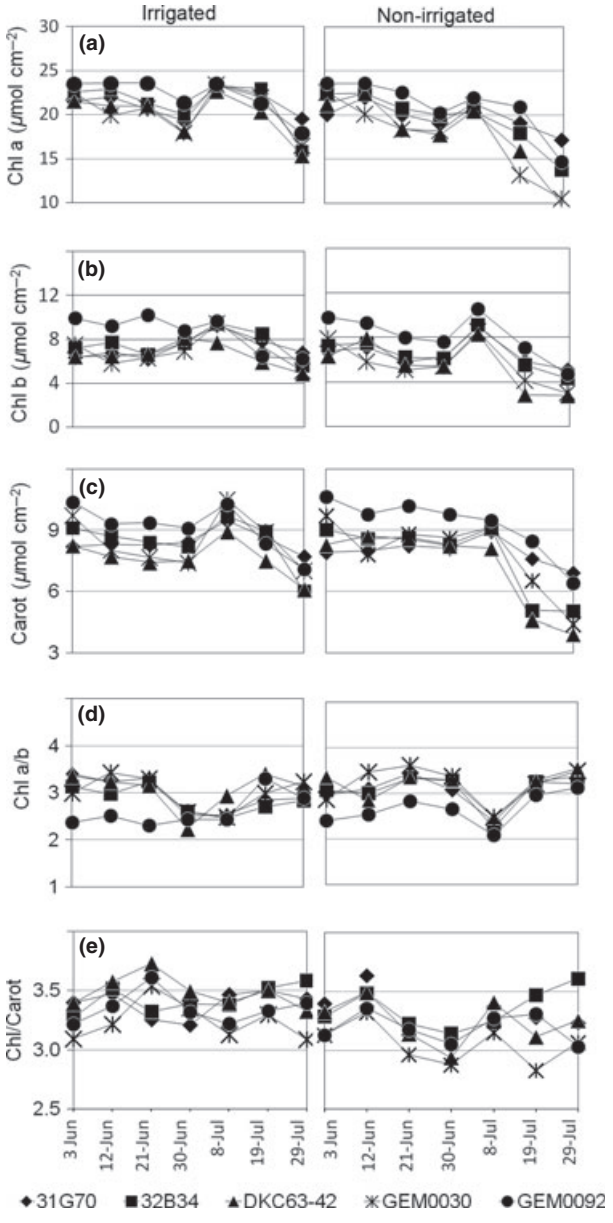

Fig. 4 Changes in (a) chlorophyll *a* (Chl *a*), (b) chlorophyll *b* (Chl *b*), (c) carotenoids (Carot), (d) chlorophyll *a*/chlorophyll *b* ratio (Chl *a*/*b*) and (e) chlorophyll/carotenoid ratio (Chl/Carot) in leaves of five corn genotypes sampled from anthesis through late dent stage under irrigated and non-irrigated conditions. Data shown are from the 2010 season because of treatment by year interaction. Samples were taken from eight plants from each genotype.

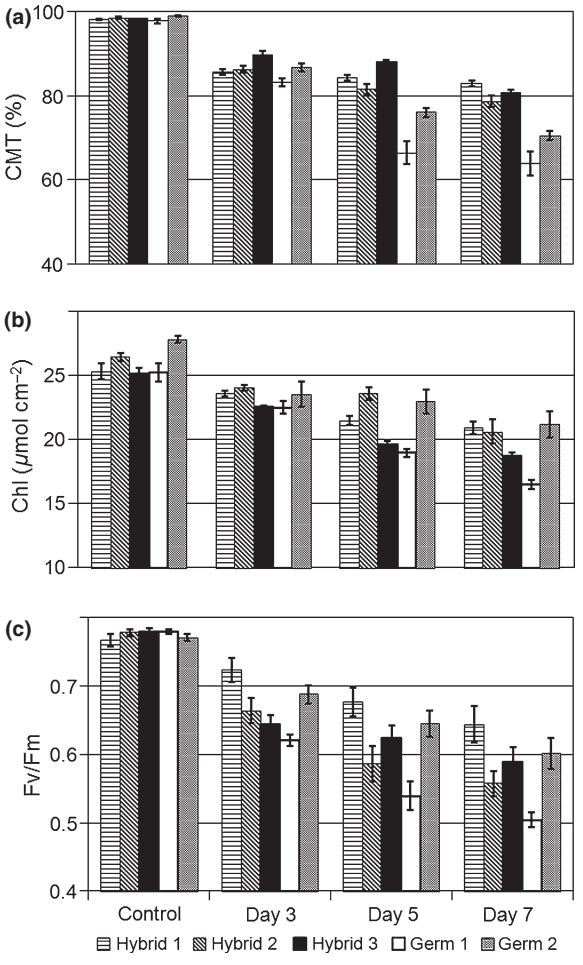

Fig. 5 Effect of *in vivo* heat stress (38/33 °C day/night temperature for 7 days) on (a) cell membrane thermostability (CMT), (b) chlorophyll content (Chl) and (c) maximum photochemical efficiency of photosystem II (Fv/Fm) in 6-week old plants of five corn genotypes under greenhouse conditions. The same heat-stressed leaves were used for CMT, Chl and Fv/Fm determination. Error bars indicate standard error (n = 6).

(Fig. 5a,b,c). Significant differences were observed among the corn genotypes in CMT ($P < 0.05$). Cell membrane injury caused by heat stress was less in the commercial hybrids than in the germplasm lines. After 5 days of treatment, Germ 1 and Germ 2, particularly Germ 1, had drastic reduction in CMT (Fig. 5a). Among the hybrids, Hybrid 3 had significantly higher CMT on Day 3 and Day 5 than the other hybrids; however, Hybrid 1 had a more gradual reduction in CMT, and by the end of the 7-day heat stress treatment, it had the highest CMT. Similar results were also observed with *in vitro* heat stress treatments by exposing leaf discs of field-grown plants for 6 h at 40 °C (Fig. 6) and for 1 h at 50 °C (data not shown). In the 40 °C treatment, it took <4.5 h for the cell membrane in the germplasm lines, particularly Germ 1, to reach 50 % relative injury, whereas in the two commercial hybrids, Hybrid 1 and Hybrid 3, that level of injury was not reached by the end of the 6 h treatment, as shown in Figure 6. This supports the results from the *in vivo* heat stress treatment on the greenhouse plants.

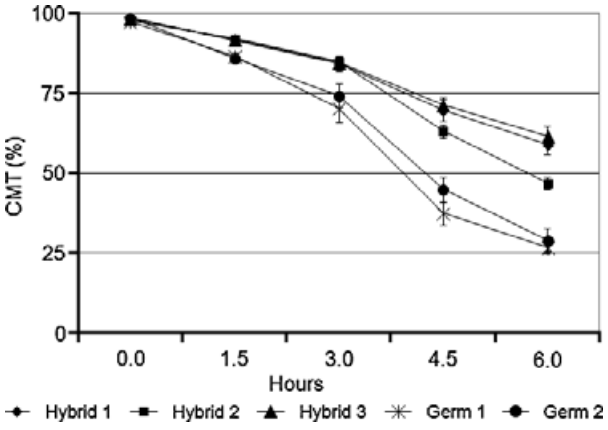

Fig. 6 Cell membrane thermostability in leaf discs from five field-grown corn genotypes incubated in deionized water at 40 °C for 6 h. Electrical conductivity measurements were made every 90 min. Error bars indicate standard error (n = 6).

Exposure of the corn plants to heat stress showed a gradual reduction in Fv/Fm during the course of the 7-day treatment as a result of a decrease in chlorophyll content (Fig. 5b,c). Similar to the CMT results, reduction in Fv/Fm was much faster in Germ 1 than in the other genotypes. In Germ 2, Fv/Fm was comparable to that of the commercial hybrids. Hybrid 1 had significantly higher Fv/Fm ($P < 0.05$) among all the genotypes throughout the 7-day heat stress treatment (Fig. 5c).

Discussion

Changes in physiological measurements corresponded well with the data obtained using the microcontroller system. Leaf water potential was significantly lower in plants grown in non-irrigated plots than those in the irrigated plots ($P < 0.01$). The driving force for water movement in the soil-plant system is the water potential gradient that exists from soil to plants and from plants to air, gradient being the highest in the soil and the lowest in the air, known as the soil–plant–air continuum (Lambers et al. 2008). As shown in Figure 3a,b, water potential in the soil was about ten times higher than in the leaves (with average values of −0.04 and −0.17 MPa in the soil and −1.5 and −1.8 MPa in the leaves in the non-irrigated and irrigated treatments, respectively). Soil water potential was about four times less in the non-irrigated plots than in the irrigated plots. There was a progressive reduction in SWP in the non-irrigated plots with little precipitation throughout the month of June for both years, and extending into July in 2010 with further reduction in SWP. However, heat stress confounded the effect of the soil moisture treatments. A gradual reduction was observed in Ψ_w in the irrigated plots, even though there was enough moisture available in the soil for the plants to maintain higher Ψ_w. High air temperature in June and early July in both years and the latter part of July in 2010, in combination with little precipitation, might have caused a higher rate of transpiration than the rate at which the roots take up water from the soil, resulting in a decline in Ψ_w. Similar studies on other crops showed similar changes in Ψ_w and its components upon exposure to heat, even though the soil water supply and relative humidity conditions were optimal, implying an effect of heat stress on root hydraulic conductance (Mazorra et al. 2002, Morales et al. 2003, Wahid et al. 2007). The non-irrigated plants were under a combination of much higher leaf moisture deficit (as low as −2.3 MPa) and heat stress, which could make it more difficult for the plants to meet the atmospheric evaporative demand and cool the leaves. This resulted in higher canopy and air temperatures in the canopy microclimate in these plots than in the irrigated plots. Differences were observed among the genotypes in these measurements. Germ 1, Germ 2 and Hybrid 3 had higher CT among the genotypes. There could be several factors that contributed to this increase in temperature, which include physiological responses and plant morphology.

Results from the *in vivo* and *in vitro* heat stress treatments on the greenhouse and field-grown plants showed that the germplasm lines were more sensitive to high-temperature stress than the commercial hybrids in terms of CMT. The structures and functions of biological membranes are heat-sensitive, as heat stress alters the tertiary and quaternary structures of membrane proteins, enhancing permeability of the membranes (Wahid et al. 2007). Thylakoid membrane and PSII are considered the most heat-labile and moisture-deficit-sensitive structures in the chloroplasts (Ristic et al. 2008, Tang et al. 2007, Sainz et al. 2010). Results from this study suggest that heat stress damage to thylakoid membrane and PSII represented by CMT (Fig. 5a) may have resulted in loss of Chl (Fig. 5b), which in turn reduced Fv/Fm (the maximum quantum efficiency of PSII) (Fig. 5c) over the course of the 7-day heat stress treatment. Germ 1, with the greatest cell membrane damage, had the lowest Chl and Fv/Fm values. However, even though Germ 2 showed more membrane damage than the hybrids, its Fv/Fm values were comparable to those of the hybrids. Its high Chl, which could be attributed to its greater specific leaf weight, may have helped in compensating for the reduction in Fv/Fm. On the other hand, the CMT determination suggested that Hybrid 1 had the most stable cell membrane by the end of the heat stress treatment resulting in higher Chl and Fv/Fm measurements than in all the other genotypes. Under both *in vivo* and *in vitro* heat stress treatments, Hybrid 3 had CMT similar to Hybrid 1.

However, preliminary data showed that its CMT was lower than that of the other two hybrids under a combination of heat and moisture deficit stress under field conditions. Previous studies suggested that the effect of a combination of the two stresses on plants is very different from the effect of each individual stress (Rizhsky et al. 2002, Prasad et al. 2011). Rizhsky et al. (2002) showed that drought stress resulted in the suppression of respiration and photosynthesis, whereas heat stress resulted in the enhancement of respiration, but did not significantly alter photosynthesis. A combination of drought and heat stress resulted in the closure of stomata, suppression of photosynthesis, enhancement of respiration and increased leaf temperature (Rizhsky et al. 2002).

Earlier studies in corn reported that large water deficits, Ψ_w lower than −1.5 MPa (Alberte and Thornber 1977), and heat stress cause loss of chloroplast membrane integrity resulting in reduction in chlorophyll content (Ristic et al. 2008). Similarly, in the present study, reductions in Chl and carotenoid contents were observed in the corn plants under the two soil moisture treatments because of a combination of these stresses. Loss of chlorophyll during drought and heat stress can be attributed to loss of pigment in the light-harvesting Chl *a*/*b* protein, consequences of which are elevated Chl *a*/*b* ratios (Alberte and Thornber 1977). The decrease in light absorption by decreasing these light-harvesting chlorophyll proteins is an essential protection mechanism of chloroplasts, which allows them to survive under unfavourable conditions by avoiding photo-oxidation and photo-inhibition (Camejo et al. 2005). In this study, the Chl *a*/*b* ratio was significantly higher and Chl/Carot ratio was significantly lower in plants in the non-irrigated plots, indicating a greater level of stress damage compared to those in the irrigated plots. There was greater reduction in Chl than in carotenoids, as shown by the lower Chl/Carot ratio, because carotenoids function in protecting the chloroplast from stress-induced oxidative damage (Young 1991, Balouchi 2010). Higher Chl *a*/*b* and lower Chl/Carot ratios detected in Germ 1 suggest that it was under severe stress and the chloroplasts were either adjusting to the condition by reducing light harvesting and/or the stress damaged the light-harvesting proteins. The lowest Chl *a*/*b* ratio observed in Germ 2 plants was not associated with stress response, as it was also shown by measurements under non-stress conditions in both field and greenhouse tests.

Morphological traits could also have contributed to how these corn plants responded to heat and moisture deficit stress. Leaf morphology plays a role in keeping the temperature and moisture balance between plants and their environment, particularly stomata being the major control points for plant water relations. Stomatal frequency in the leaves of Germ 1 and Germ 2 was lower than in the commercial hybrids under field conditions (data not shown). This could reduce the rate of transpiration in these genotypes, which could result in increased canopy temperature and the temperature of the air around the plants. A greenhouse study also showed that stomatal conductance and transpiration rate were significantly lower in these genotypes compared to that of the hybrids (data not shown). In addition, Germ 1 is smaller in size than the other genotypes followed by Germ 2 and Hybrid 3 (Table 3). Because of less ground cover by these plants, higher soil temperature in these plots might have been caused by solar radiation, which in turn could have contributed to the increase in canopy and air temperature.

As evidenced by the reduction in the photosynthetic apparatus in the irrigated treatments, heat stress, which also indirectly could cause moisture deficit stress, appears to be the factor that affected performance of the corn genotypes in the irrigated plots. But the combined effect of heat and severe moisture deficit stress had a much greater impact in the non-irrigated plots. The higher sensitivity of Germ 1 and Germ 2 to heat stress is perhaps not surprising because of their genetic background. These germplasm lines have tropical background, Germ 1 with 50 % background from Brazil and Germ 2 with 25 % background from Cuba. Even though the tropical climate is warm, these germplasm lines may not have been exposed to the extent of heat that occurs in the Mississippi Delta region during the months of June, July and August. The commercial hybrids, however, were developed for this environment, and they seem to be more thermostable than these germplasm lines. This study suggests that Hybrid 1 was the most heat and drought stress tolerant among all the genotypes. It showed the least cell membrane damage and the lowest canopy temperature under irrigated and non-irrigated treatments. This commercial hybrid is known to have good tolerance to drought stress and is widely grown in the Mississippi Delta region.

Our results demonstrated that the low-cost microcontroller-based monitoring system that we developed to monitor moisture deficit and heat stress, in combination with physiological measurements, was effective in evaluating difference in corn genotypes in response to these stresses. Continuous data collection is advantageous particularly in monitoring CT, which is subject to rapid fluctuations over the course of a day, and the associated factors such as air temperature and soil moisture. In this study, variations observed in air and soil temperature and soil moisture in plots of the individual corn genotypes helped explain the variation in CT among genotypes, and these variations were also reflected in physiological responses. Under field conditions, as reduced water

availability is frequently associated with high-temperature stress, this automated system would be useful in evaluating crop plants under these stress conditions. As there are multiple environmental stress factors on plants under natural conditions, monitoring these factors and relating the information to the plants' physiological and molecular responses is a realistic approach to develop plants with enhanced stress tolerance.

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